THE DETECTION OF β-OXYBUTYRIC ACID IN THE URINE.

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In a considerable number of diseases, notably in diabetes mellitus, the detection of β-oxybutyric acid is of great interest and of importance both from a diagnostic and therapeutic standpoint. The statement, repeated in a number of text-books on chemical diagnosis, to the effect "that when a urine, which has been fermented, deviates the plane of polarized light to the left, the presence of β-oxybutyric acid may be inferred," is certainly hesitating enough to leave some doubt in our minds as to this mode of detection. This doubt is augmented when we review the list of substances at times found in the urine which may produce the same effect. Among those most frequently encountered may be mentioned creatinin and various compounds of glycuronic acid. Also certain compounds of lead, which are formed when lead acetate is added in the process of clearing urine for examination in the spectroscope, have been shown by Magnus-Levy[1] to possess a marked levorotatory action.

It is not for lack of methods for the detection of β-oxybutyric acid that I venture to suggest a new one. Procedures have been worked out by a large number of investigators—Tollens,[2] Wolpes,[3] Naunyn,[4] Stadelman,[5] Magnus-Levy,[6] Bergell,[7] Darmstäder,[8] Waldvogel,[9] Shaffer,[10] and Black.[11] Some of these methods are adapted to the quantitative as well as the qualitative determinations of β-oxybutyric acid, some of them have serious defects, practically all are laborious chemical procedures which require many hours of work, the facilities of a well-equipped laboratory, and the manipulations of a skilled chemist. Parenthetically it may be said that the methods recently proposed by Shaffer and by Black have added much to our facility in determining oxybutyric acid and appear to be very accurate.

The method which is here presented is exceedingly simple, requires little time, needs no elaborate chemical apparatus, and can be applied by any general practitioner. It depends upon the following principles: The removal of acetone and diacetic acid by heat, the oxidation of β-oxybutyric acid to acetone by means of hydrogen peroxide, and the detection of acetone in the urine thus treated by means of Lange's test.[12]

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[1] Arch. f. exp. Path. u. Pharm., 1899, Band xlii.

[2] Ann. d. chem., 1885, p. 228.

[3] Arch. f. exp. Path. u. Pharm., 1886, p. 21.

[4] Noth. Spec. Path. u. Ther., Band vii, 1, 181.

[5] Ueber den Einfluss de Alkalien auf d. mensch. Stoffer, Stuttgart, 1890.

[6] Arch. f. exp. Path. u. Pharm., 1901, Band xlv

[7] Ztschr. f. physiol. Chem., 1901, Band xxxiii.

[8] Ibid., 1903, i, 37.

[9] Die Acetonkorper, Stuttgart, 1903, p. 38.

[10] Jour. Biol. Chem., 1908, v, 211.

[11] Ibid., 1908, v, 207.

By boiling the preformed acetone, diacetic acid and the volatile fatty acids are removed from the urine; the boiling must be continued long enough completely to remove all of these bodies, and the urine after such treatment should give a negative result with Lange's test. This test depends upon the property of urine containing acetone to develop a purplish red color when treated with glacial acetic acid, a few drops of a watery solution of sodium nitroprussiate, and ammonia. The reaction is not given with alcohol or aldehyde; creatinin when present in considerable amounts gives a brownish color, which, however, may be distinguished from the purplish red color of the acetone reaction.

To the urine from which acetone, diacetic acid, and the volatile fatty acids have been removed, but which still retains its β-oxybutyric acid, peroxide of hydrogen is added. By this means the β-oxybutyric acid, as has been shown by Dakin,[13] is oxidized to acetone. It may be noted that this cannot be a quantitative method, even in a rough degree, since Dakin has shown that the end-products of the oxidation of β-oxybutyric acid by peroxide of hydrogen consist of different substances varying in amount according to the course which the oxidation process takes, and is never acetone alone.

An abbreviated outline of the chemical process involved may be represented by these formulæ:

$$\overset{\beta\text{-oxybutyric acid.}}{CH_3-CHOH-CH_2-COOH} + O = \overset{\text{Diacetic acid.}}{CH_3-CO-CH_2-COOH} + H_2O$$

$$\overset{\text{Diacetic acid.}}{CH_3-CO-CH_2-COOH} = \overset{\text{Acetone.}}{CH_3-CO-CH_3} + \overset{\text{Carbon dioxide.}}{CO_2}$$

In the course of a study of the acetone bodies extending over a number of years I have never found β-oxybutyric acid in the urine unless a strong Arnold's[14] reaction and a strong ferric chloride reaction (Gerhardt's) were present. To a urine showing these reactions the test for β-oxybutyric acid may be applied in the following manner:

To 20 c.c. of the suspected urine add 20 c.c. of water and a few drops of acetic acid and boil until the volume is reduced to about 10 c.c. To this residue add water to the original volume (*i. e.*, until it measures 20 c.c.). Put this into two test-tubes (B and C) of equal size, 10 c.c. in each test-tube. To one of the test-tubes (C) add one cubic centimeter of peroxide of hydrogen, warm gently for about one minute (do not boil), and then allow the fluid to cool. Add to each test-tube (B and C) one-half of one cubic centimeter of glacial acetic acid and a few drops of a freshly prepared watery solution of sodium nitroprussiate and mix. Overlay the solution in each test-tube with 2 c.c. of concentrated ammonium hydroxide. Allow these to stand for four or five hours, and at the end of this time compare the two test-tubes. At the point of contact between the ammonia and the underlying fluid B will show no ring (or a faint brown ring if much creatinin is present), test-tube C, to which hydrogen peroxide was added, will show a purplish red contact ring if β-oxybutyric acid was originally present; if β-oxybutyric acid was not present, the two test-tubes will not differ in appearance. If the two test-tubes are now shaken, the difference in color will be seen throughout the fluid; this difference is intensified by allowing the tubes to stand for fifteen or twenty minutes after shaking.

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[12] Münch. med. Woch., 1906, Band liii, p. 1764.

[13] Jour. Biol. Chem., 1908, iv, 77.

[14] Archives of Internal Medicine, February, 1908.

To detect slight differences in the color of the rings, the two test-tubes should be held side by side against a white background while the observer stands with his back to the light.

The oxidation by means of hydrogen peroxide is gradual and reaches its maximum in about four or five hours, after which the color slowly fades. When a very large amount of β-oxybutyric acid is present the difference in the two test-tubes may become evident in a few minutes. It is always well to prepare the two test tubes as above for comparison. B will show whether all preformed acetone and diacetic acid have been driven off, and will also show the creatinin ring (if present), so that its color may be compared with the color of the ring in tube C.

The test may be applied to the urine either before or after fermentation; the presence of sugar does not interfere with the reaction. If albumin is present it should be removed by filtration after the urine has been boiled.

The method is moderately delicate and will certainly detect β-oxybutyric acid when present to the extent of 0.3 per cent. and probably less.

The following table shows a number of urines in which the test, as above described, showed a positive reaction for β-oxybutyric acid in which the acid was quantitatively determined by Shaffer's method.

Specimen number.	Levorotation after fermentation.	Quantity in 24 hours, c.c.	β-oxybutyric acid.		
			Total grams.	Grams per liter.	Per cent.
1	0.7	4050	26.60	6.6	0.66
2	0.7	2310	11.94	5.2	0.52
3	0.5	1800	10.82	5.1	0.51
4	0.6	1800	9.15	5.0	0.50
5	0.5	4650	21.67	4.7	0.47
6	0.5	2550	8.01	3.1	0.31
7	0.2	3360	8.68	2.6	0.26

That the test is somewhat delicate may be inferred from the fact that I have obtained it in a number of diabetic urines with a levorotation after fermentation of 0.2 and a ferric chloride reaction much weaker than that given by any of the above urines in which the oxybutyric acid was quantitatively determined. It has been my experience that the amount of β-oxybutyric acid varies directly (although not necessarily proportionately) with the amount of diacetic acid present and that the ferric chloride reaction varies directly in intensity with the amount of diacetic acid present.[15]

In a number of normal urines tested the reaction has not been obtained. It was also absent in a number of urines showing a weak ferric chloride reaction.

THE EFFECT OF CERTAIN SO-CALLED MILK MODIFIERS ON THE GASTRIC DIGESTION OF INFANTS.

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In the fall of 1907, at the instigation of Dr. L. Emmett Holt, investigations were undertaken at the Rockefeller Institute in New York, in the hope of throwing light on some of the as yet obscure questions of infant digestion. The problem to be attacked was that of the digestion of milk in the infant's stomach, and more particularly the exact action of the more commonly used, so-called "milk modifiers," and their influence upon digestion both in health and disease.

A review of the literature of the gastric digestion of the infant[1] shows much valuable work on its physiology and its variations according to age and health. It has been shown that all the physiological factors occurring in the adult stomach are present in the infant, but in weaker form. It has further been proved that the younger the infant, the more active the motility of the stomach, that this motility is greater with woman's milk than with artificial food, and in health than in disease. It has been proved that the acidity of the gastric contents increases regularly after a meal for some time, but that free hydrochloric acid cannot be demonstrated for an hour or more after the ingestion of the food. This phenomenon has been shown to be due not to a lack of secretion of hydrochloric acid during the early stages of digestion, but to the striking power which

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[15] Archives of Internal Medicine, February, 1908.

[1] Amer. Jour. Med. Sci., 1909, cxxxvii, 674.